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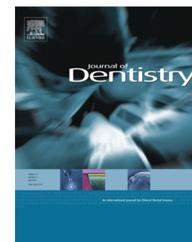

Effect of autogenous and fresh-frozen bone grafts on osteoblast differentiation

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ABSTRACT

Objective: Fresh-frozen bone allograft (FFBA) is an alternative to autogenous bone (AB) for reconstructing maxillary bone. Despite the promising clinical results, cell responses to FFBA and AB were not evaluated. Thus, our aim was to compare cells harvested from maxillary reconstructed sites with either AB or FFBA in terms of osteoblast differentiation and to evaluate the effect of culturing cells in contact with FFBA.
Methods: Cells harvested from three patients submitted to bilateral maxillary reconstruction with AB and FFBA were cultured to evaluate: proliferation, alkaline phosphatase activity, extracellular matrix mineralization and gene expression of osteoblastic markers. The effect of FFBA on osteoblast differentiation was studied by culturing cells harvested from AB in contact with FFBA and evaluating the same parameters. Data were compared using either two-way ANOVA followed by Tukey-*b* test or Student's *t* test ($p \leq 0.05$).
Results: Cell proliferation was higher in cultures from AB grafted sites and extracellular matrix mineralization was higher in cultures derived from FFBA grafted sites. The gene expression of alkaline phosphatase, RUNX2, bone sialoprotein and osteocalcin was higher in cells derived from FFBA compared with cells from AB grafted sites. However, the exposure of cells derived from AB to FFBA particles did not have any remarkable effect on osteoblast differentiation.
Conclusions: These results indicate the higher osteogenic activity of cells derived from FFBA compared with AB reconstructed sites, offering an explanation at cellular level of why FFBA could be a suitable alternative to AB for reconstructing maxillary bone defects.
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1. Introduction

Oral rehabilitation using dental implants is a well-established therapy with a high long-term success rate, which relies on the quality and amount of bone tissue. Insufficient bone volume represents a challenging clinical situation in the implantology field in terms of functional and aesthetic parameters.[1] The need of maxillary reconstructions to retrieve the bone volume prior to the implant placement can be achieved by several procedures including the use of grafts such as autogenous bone (AB), allogenous bone and alloplastic materials.[2–5] The

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histocompatibility, osteoinductive and osteoconductive properties make AB the gold standard graft; however, the amount of bone, donor site morbidity and unpredictable graft resorption are limitations of using this bone source.[6–9] Biomaterials such as the anorganic bovine bone, which exhibits very similar physicochemical properties of the human bone, induce a delay in bone formation and exert negative effects on osteoblast differentiation.[10–12] In this context, different grafts have been tested and fresh frozen bone allograft (FFBA) may represent an alternative to reconstruct bone defects.

The FFBA is aseptically harvested from different skeletal sites of live or cadaveric donors, immediately frozen and stored at −80 °C.[13] The rigorous protocol for bone processing, which eliminates living cells and consequently the risk of transmission of diseases, and the reduced immunological reaction to the graft have increased the clinical and scientific interest in the FFBA.[13–16] The FFBA acts as a scaffold allowing the ingrowth of cellular and vascular components, and ultimately promoting the bone tissue regeneration.[17] Some studies have shown that the use of dental implants in maxillary defects reconstructed with FFBA is a reliable technique that may be safely used as an alternative to AB graft.[18–20] Despite the promising clinical and histological findings, up to now, there are no studies investigating the FFBA behaviour at the cellular level. Thus, the aim of this paper was to compare cells harvested from maxillary reconstructed sites with either AB graft or FFBA in terms of osteoblast differentiation. Additionally, to eliminate the influence of *in vivo* micro-environment and to evaluate the effect of FFBA itself on cells, we cultured osteoblasts harvested from AB in direct contact with FFBA.

2. Materials and methods

2.1. *Osteoblast differentiation of cells derived from maxillary reconstructed sites with either AB graft or FFBA*

2.1.1. *Patient selection*

The Committee of Ethics in Research of the School of Dentistry approved the procedures and all patients signed the informed consent. Three healthy female patients, totally edentulous, with an average age of 55.5-year-old (ranging from 52 to 62-year-old), were selected as subjects for a clinical trial to compare AB graft with FFBA and submitted to bilateral maxillary verticosagittal reconstruction surgery. The left and right sides were randomly reconstructed with either AB graft derived from mandibular ramus or FFBA harvested from femoral heads (Musculoskeletal Tissue Bank of Marilia Hospital – Unioss, Marilia, SP, Brazil). After 6 months, punch biopsies were obtained from each reconstructed site immediately before the dental implant (Neodent, São Paulo, SP, Brazil) placement. The bone fragments from the two reconstructed sites, AB and FFBA, of the three patients were processed as described below.

2.1.2. *Isolation and cell culture*

The osteoblastic cells were isolated from bone fragments of the AB and FFBA reconstructed sites by enzymatic digestion using collagenase type II (Invitrogen, Carlsbad, CA, USA) and expanded in α-minimum essential medium (Invitrogen), supplemented with 10% foetal bovine serum (Invitrogen), 50 μg/mL gentamicin (Invitrogen), 0.3 μg/mL fungizone (Invitrogen), 10^{-7} M dexamethasone (Sigma–Aldrich, St Louis, MO, USA), 5 μg/mL ascorbic acid (Invitrogen), and 7 mM β-glycerophosphate (Sigma–Aldrich). First passage cells were cultured in 24-well culture plates (Falcon, Franklin Lakes, NJ, USA) at a cell density of 2×10^4 cells/well for periods of up to 21 days. The cultures were incubated at 37 °C in a humidified atmosphere of 5% CO_2 and 95% air and the medium was changed every 3 days.

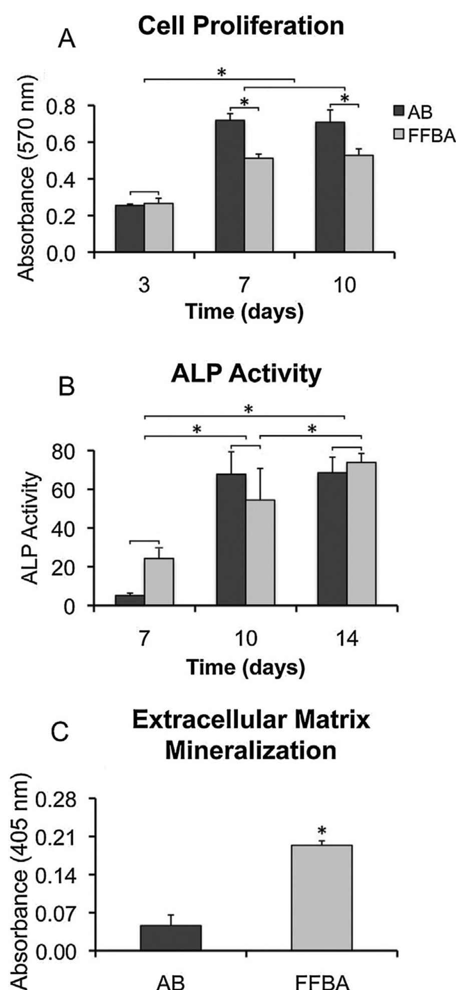

Fig. 1 – Proliferation (A), alkaline phosphatase (ALP) activity (B), and extracellular matrix mineralization (C) of cells derived from autogenous bone (AB) graft and fresh-frozen bone allograft (FFBA) reconstructed sites. The cell proliferation was higher ($p = 0.001$) in cultures from AB compared with FFBA grafted sites at days 7 and 10. The ALP activity was statistically the same ($p = 0.818$) in cultures from AB and FFBA grafted sites at all evaluated time points. At day 21, the calcium content in extracellular mineralized matrix was higher ($p = 0.001$) in cultures derived from FFBA grafted sites compared with AB-derived ones. Data are presented as mean ± standard deviation ($n = 5$). Asterisks indicate statistically significant differences ($p \leq 0.05$).

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